High-Density Lipoprotein Cholesterol (HDL-C) Assay Kit

Catalog No.: abx298880

Size: 96 tests

Detection Range: 0.06 mmol/L – 3.8 mmol/L

Sensitivity: 0.06 mmol/L

Storage: Store all components at 4°C in the dark.

Application: For detection of high-density lipoprotein cholesterol (HDL-C) concentration in serum and plasma samples.

Introduction

Abbexa's High-Density Lipoprotein Cholesterol Assay Kit is a quick, convenient, and sensitive method for measuring and calculating high-density lipoprotein cholesterol concentration. High-density lipoprotein forms a soluble compound under the action of surfactants. The particles are therefore able to directly react with reagents containing cholesterol esterase (CE) and cholesterol oxidase (CO) which catalyze HDL-C to produce hydrogen peroxide. The hydrogen peroxide can then be catalyzed by peroxidase (POD) in the presence of 4-aminoantipyrine (4-AA) and phenol (T-OOS) to form a red quinone compound with an absorbance maximum of 546 nm. The intensity of the color is proportional to the high-density lipoprotein cholesterol (HDL-C) concentration, which can then be calculated.

Kit components

1. 96-well microplate
2. Enzyme Working Solution 1: 18 ml
3. Enzyme Working Solution 2: 6 ml
4. Standard: 1 vial
5. Plate sealer: 2

Materials required but not provided

1. Microplate reader (546 nm) or Biochemical analyzer (546 nm)
2. Double-distilled water
3. PBS (0.01 M, pH 7.4)
4. Normal saline (0.9 % NaCl)
5. Pipette and pipette tips
6. 1.5 ml microcentrifuge tubes
7. Centrifuge
8. Vortex mixer
9. Incubator
10. Water bath

Protocol

A. Preparation of samples and reagents

1. Samples

Isolate the test samples soon after collecting and analyze immediately or aliquot and store at -80°C for long-term storage. Avoid multiple freeze-thaw cycles.

The following sample preparation method is intended as a guide and may be adjusted as required depending on the specific samples used.

- **Serum** and **Plasma:** Serum and plasma samples can be tested directly. Unused sample can be stored at -80°C for up to one month.

It is recommended to carry out a preliminary experiment to determine the optimal dilution factor of samples before carrying out the formal experiment. Dilute samples into different concentrations with normal saline (0.9 % NaCl) or PBS (0.01 m, pH 7.4), then carry out the assay procedure. The recommended dilution factor for different samples is as follows (for reference only):

Sample Type	Dilution Factor
Human serum	1
Human plasma	1
Mouse serum	1
Mouse plasma	1
Rat serum	1
Rat Plasma	1
Porcine serum	1

Note:

- Fresh samples or recently obtained samples are recommended to prevent degradation and denaturalization that may lead to erroneous results.

2. Reagents

- **Enzyme Working Solution 1 and 2:** Incubate the amount needed for the assay of Enzyme Working Solution 1 and Enzyme Working Solution 2 at 25°C for 15 minutes. The remaining Enzyme Working Solution 1 and Enzyme Working Solution 2 can be stored at 4°C.
- **Standard Solution:** Dissolve 1 vial of standard with 200 µl of double-distilled water. Unused Standard Solution can be stored at 4°C for up to 2 weeks in the dark.

Note:

- Allow all reagents (except Enzyme Working Solution 1 and 2) to equilibrate to room temperature before use.
- Take care to avoid bubbles when adding the liquid to the microplate.
- Avoid contamination of reagents with glucose, cholesterol, etc.
- The amount of reagent and sample can be adjusted proportionally according to the volume of the cuvette.

B. Assay Procedure

Pre-heat the incubator and ensure it has reached a stable temperature before use

96 Well Microplate Reader:

1. Assign microplate wells for each standard, sample, and blank. Each sample requires a corresponding blank. *It is strongly recommended to prepare all the tubes in duplicate.*
2. Add 5 µl of double-distilled water to the blank wells.
3. Add 5 µl of Standard Solution to the standard wells.
4. Add 5 µl of Sample to the sample wells.
5. Add 180 µl of Enzyme Working Solution 1 to all wells.
6. Mix fully, then incubate at 37°C for 5 minutes.
7. Measure the OD (A_1) of each well with a microplate reader at 546 nm.
8. Add 60 µl of Enzyme Working Solution 2 to all wells.
9. Mix fully, then incubate at 37°C for 5 minutes.
10. Measure the OD (A_2) of each well with a microplate reader at 546 nm

Biochemical Analyzer:

1. Set up the biochemical analyzer using the following parameters:
    - Main Wavelength: 546 nm
    - Reaction Type: Terminal Method
    - Reaction Direction: Up (+)
2. Add 5 µl of sample or double distilled water to the tube.
3. Add 180 µl of Enzyme Working Solution 1 to the tube. Mix fully.
4. Incubate at 37°C for 5 minutes. Measure the OD value at 546 nm using the biochemical analyzer. Record this as A_1.
5. Add 60 µl of Enzyme Working Solution 2 to the tube. Mix fully.
6. Incubate at 37°C for 5 minutes. Measure the OD value at 546 nm using the biochemical analyzer. Record this as A_2.

$$\Delta A = A_2 - A_1$$

C. Calculation of Results

Plot the standard curve, using the OD of the standard dilutions (adjusted for the blank) on the y-axis, and their respective concentrations on the x-axis. The curve should form a straight line, described by the formula $y = ax + b$. Based on this curve, the concentration of low-density lipoprotein cholesterol in each sample well can be derived with the following formulae:

1. Serum (Plasma) and other liquid samples:

Calculations from microplate reader:

$$\mathbf{HDL\text{-}C\ (mmol/L) = F \times C \times \frac{\Delta A_{Sample} - \Delta A_{Blank}}{\Delta A_{Standard} - \Delta A_{Blank}}}$$

Calculations from biochemical analyzer:

$$\mathbf{HDL\text{-}C\ (mmol/L) = F \times C \times \frac{\Delta A_{Sample}}{\Delta A_{Standard}}}$$

where:

ΔA_{Sample}	ΔA value of sample
$\Delta A_{Standard}$	ΔA value of standard
ΔA_{Blank}	ΔA value of blank
C	Concentration of standard
F	The dilution factor of sample

Technical Support

For troubleshooting and technical assistance, please contact us at support@abbexa.com.